LOCOMOTOR ACTIVITY RHYTHMS OF JUVENILE ATLANTIC SALMON (*SALMO SALAR*) IN VARIOUS LIGHT CONDITIONS

NANCY E. RICHARDSON AND JAMES D. McCLEAVE

Department of Zoology, University of Maine, Orono, Maine 04473

Endogenous circadian components in the activity rhythms of fishes have not been convincingly demonstrated. Various circadian oscillations, subject to phase setting primarily by natural or artificial light cycles, have been documented in several fishes. This synchronizing effect of light cycles on the locomotor activity patterns of salmonids, in particular, has been cited in several instances (Ali, 1964; Swift, 1964; Byrne, 1968; Varanelli and McCleave, 1974).

Whereas circadian rhythms in certain mammals and birds have been known to free-run in constant conditions for months at a time (Aschoff, 1960, 1966), the rhythms termed "endogenous" in various fish studies have usually not been apparent for more than a few days. Activity rhythms have persisted for two or three days in constant conditions in juvenile Atlantic herring, *Clupea harengus*, (Stickney, 1972), juvenile sockeye salmon, *Oncorhynchus nerka*, (Byrne, 1968), juvenile Atlantic salmon, *Salmo salar*, (Ali, 1964), and in a European minnow, *Leucaspius delineatus*, (Seigmund and Wolff, 1973). The adult sea lamprey, *Petromyzon marinus*, maintained a circadian activity rhythm for five days in constant dim light at which time observations were discontinued (Kleerekoper, Taylor and Wilton, 1961).

An exceptional case is the circadian rhythm of the swell shark, *Cephaloscyllium ventriosum*, which continued for about 15 days in both constant light and constant darkness (Nelson and Johnson, 1970). The same authors did not observe free-running rhythmicity in the horn shark, *Heterodontus francisci*.

Circadian rhythms of swimming speed in the bluefish, *Pomatomus saltatrix*, have been reported to dissipate after two or three days in constant dim light, but later to become reestablished (Olla and Studholme, 1972). A similar reestablishment of rhythmicity has been suggested for Atlantic salmon (Ali, 1964).

Gibson (1971) found that exposure to light-dark cycles was necessary for as long as two to four months before circadian rhythms which would persist in constant darkness could be entrained in blennies, *Blennius pholis*. Activity rhythms with a 12 hr period were easily entrained in the same fish by changes in hydrostatic pressure resulting from local tidal cycles.

A circadian rhythm of electric organ discharge has been observed to free-run in constant dim light in the electric gymnotid, *Gymnorhamphichthys hypostomous*, (Lissmann and Schwassmann, 1965). The appearance of the corresponding locomotor activity rhythm in these fish seems dependent upon the presence of a light-dark cycle and the natural environmental substrate. It was concluded that the activity rhythm is not a good indicator of the endogenous oscillation (Schwassmann, 1971).

Locomotor activity patterns in juvenile Atlantic salmon were observed by

Varanelli and McCleave (1974). Photoperiod and temperature were altered in different experiments to approximate concurrent seasonal conditions. Activity rhythms became synchronized to the imposed light-dark cycle, but in most cases failed to persist in constant conditions.

The present study was designed to gain more information about the effects of light regimes on the activity patterns of Atlantic salmon by subjecting larger numbers of fish to the same experimental conditions. About two thirds of the fish were simultaneously tested for their responses to weak extremely low frequency electric and magnetic fields. Since these fields were found to have no effect on activity (Richardson, McCleave and Albert, unpublished data), fish exposed to the fields are combined with control fish in this paper.

Materials and Methods

Between June 1972 and June 1973, 16 experiments involving 192 fish were carried out. Usable records were obtained from 177 fish. In each experiment the locomotor activity of 12 fish was individually recorded for 10 days, though a few fish records were less than 10 days due to equipment failures. The fish were exposed to a 12 hr light–12 hr dark cycle (LD 12:12) in eight experiments, to constant light (LL) in four experiments, and to constant darkness (DD) in three. In one experiment, a 23 hr cycle containing 1 hr of light (LD 1:22) was imposed.

Sea run Atlantic salmon parr between 13 and 20 cm total length were obtained from the Craig Brook National Fish Hatchery in East Orland, Maine. Fish were placed in individual activity chambers as soon as they arrived at the university and were allowed two days to acclimate to the apparatus.

The apparatus and recording methods were similar to those described by Varanelli and McCleave (1974). The activity chambers were circular channels 10 cm wide by 27 cm deep, built from two concentric polyethylene cylinders, 47 cm and 27 cm in diameter. Air was bubbled into the center of the inner cylinder and diffused into the channel through holes in its inner side. Two activity chambers were housed in each of six light-tight water baths. The chambers were water-tight and opaque so that fish in the same water bath had no visual or chemical contact with one another. Water was circulated through the baths from a refrigeration unit which maintained the temperature at $15 \pm 1°$ C. Temperature was continuously recorded in one of the chambers.

Activity was recorded electromechanically. Two plastic probes were suspended in opposite sides of the swim channel. The ends of the probes hung 4 cm off the bottom. The upper portion of each probe was attached to a piece of copper braid which hung through a carbon ring. When a fish moved the probe, contact was made with the carbon ring completing an electric circuit. This caused one count to be registered by an automatic counter. The two probes from each chamber were wired in parallel to one counter, which printed 10 times per hour. Counter tapes were removed once each day and the activity counts were coded directly for computer processing.

Fish were not fed during the experiments to eliminate inducement of activity cycles based on feeding. Experiments were carried out in a basement laboratory

which was entered only once each day at 1200 EDT. Efforts were made to isolate the fish from as many environmental disturbances as possible.

Light was provided by either fluorescent or incandescent bulbs mounted on the lid of each water bath. Illumination ranged between 1 and 15 lux at the water surface depending on the area of the tank measured. In the LD 12:12 experiments, automatic timers switched the lights on at 0600 EDT and off at 1800 EDT each day.

Computer drawn plots of the hourly activity for each fish over the course of each day were obtained. Composite plots encompassing all 10 days were also

TABLE I

*Summary of locomotor activity patterns and periodicities of
Atlantic salmon parr in LD 12:12*

Experiment date	Number of salmon				Range of period lengths	Mean period length
	Diurnal	Nocturnal	Light change	Aperiodic		
6/22–7/ 2/72	11	0	1	0	23.8–24.7	24.1
7/ 7–7/17/72	5	0	6	0	23.9–24.0	24.0
7/21–7/31/72	6	0	4	0	24.0–24.2	24.1
8/ 4–8/14/72	8	1	0	0	24.0	24.0
2/15–2/25/73	1	8	3	0	24.0–24.5	24.1
3/ 3–3/13/73	3	4	4	1	24.0–24.5	24.0
4/15–4/25/73	1	5	5	0	23.9–24.6	24.1
5/16–5/26/73	0	2	9	0	23.9–24.0	24.0

produced for each fish. These plots were visually examined to determine the nature of individual activity patterns. Data from fish with similar patterns were combined to produce plots illustrating typical patterns.

Activity records were analyzed for periodicity by the periodogram method of Enright (1965). This method is appropriate for serially correlated data because it does not require random independent observations. The periodogram method calculates amplitudes for a series of possible period lengths within the range of interest. The amplitude is the standard deviation of the hourly means of activity. The hourly means used for each amplitude were drawn from arrangement of the data based on the assumption that a given period existed. If a true periodicity exists the amplitude produced by the proper assumed period length will be clearly larger than the other amplitudes. Computer calculated amplitudes were obtained for assumed period lengths between 3.0 and 33.0 hr in increments of 0.1 hr.

Results

LD 12 : 12

Periodogram analysis showed that fish subjected to a 12:12 light-dark cycle possessed a rhythmicity in locomotor activity with a period length very close to 24.0 hr (23.8–24.7) (Table I, Fig. 1a). Only one fish in 88 was aperiodic. All periodic fish exhibited one of three typical patterns: diurnal, nocturnal, or active primarily at times of light-dark transitions. From all eight LD experiments
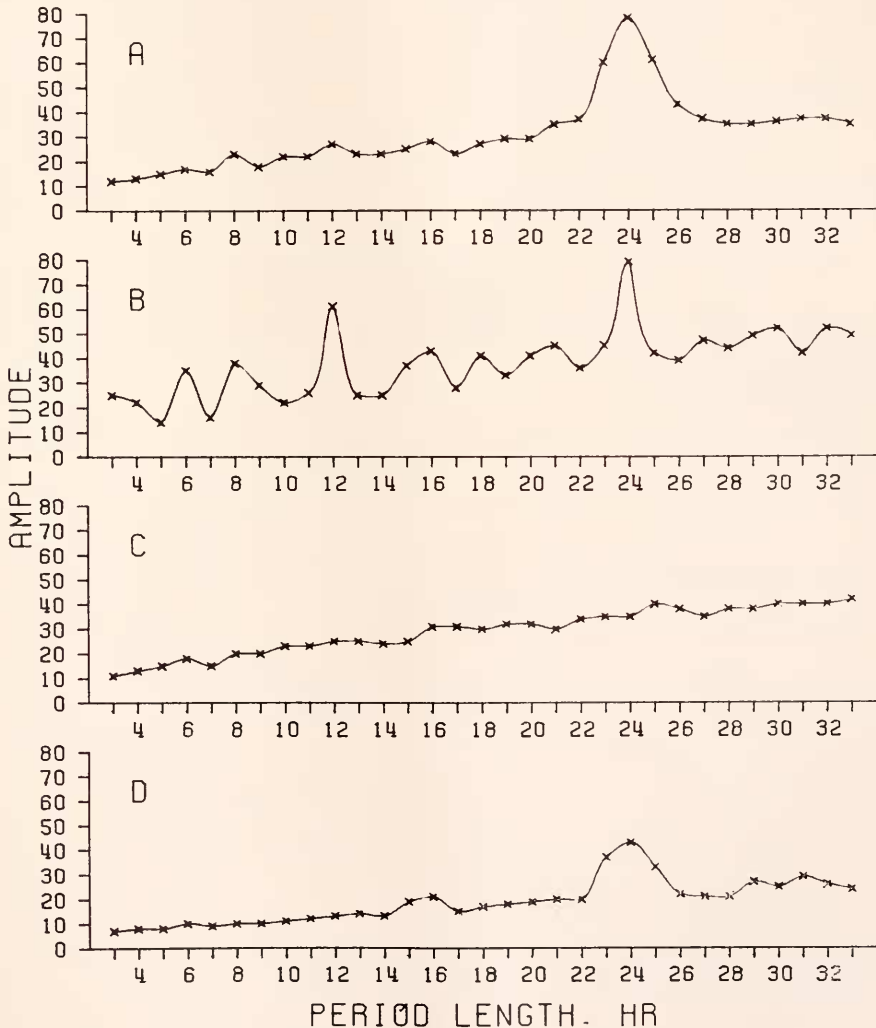

FIGURE 1. Sample periodograms for (a) a diurnal Atlantic salmon in LD 12:12, (b) a light-change-active salmon in LD 12:12, (c) an aperiodic salmon in DD, (d) salmon in DD with 24.0 hr periodicity. (Periodograms were calculated in steps of 0.1 hr, but only hourly points are plotted for clarity).

35 fish were diurnal, 20 were nocturnal and 32 were active at light change (Fig. 2). Mean percentages of daily activity occurring when lights were on ranged from 53 to 94% for diurnal fish, when lights were off from 62 to 86% for nocturnal fish, and in the 3 hr following lights on and lights off from 28 to 55% for light-change-active fish (Fig. 3). Expected percentages, if activity were equally distributed throughout the day, were 50%, 50%, and 25%, respectively, for diurnal fish, nocturnal fish, and light-change-active fish.

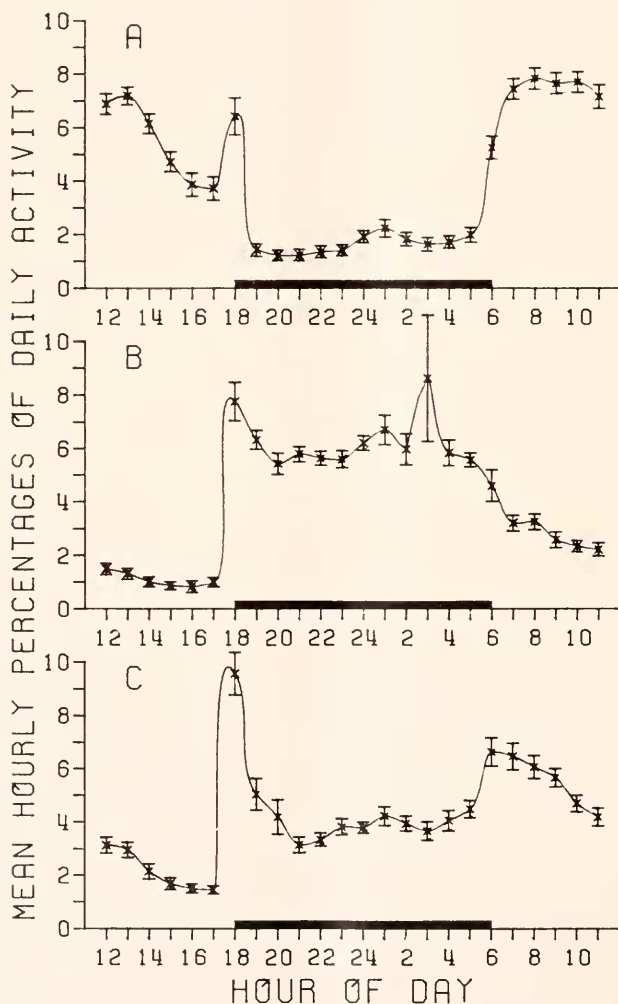In all three patterns the most abrupt increase in hourly activity occurred during

FIGURE 2. Composite plots of mean hourly percentages of daily activity ± 1 standard error for Atlantic salmon in LD 12:12 showing the three activity patterns; (a) light-active fish, N = 35, (b) dark-active fish, N = 20, (c) light-change-active fish, N = 32. Dark bar indicates lights off.
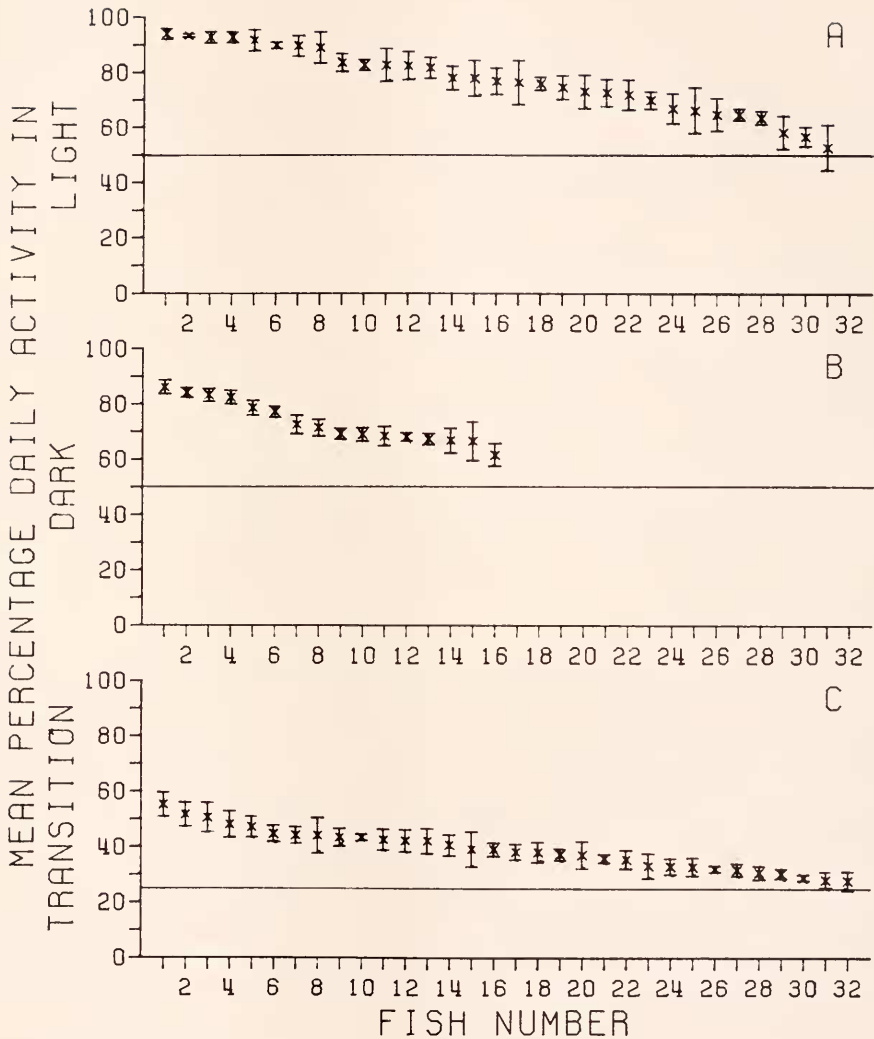
FIGURE 3. Mean percentage of daily activity ±1 standard error in LD 12:12 (a) of diurnal Atlantic salmon when lights were on, (b) of nocturnal salmon when lights were off, (c) of light-change-active salmon in the 3 hr following lights on and lights off. Only those salmon for which a full 10 day record was available were used.

the hour immediately following lights off (Fig. 2). Diurnal and light-change-active fish showed a second burst of activity in the hour following lights on. Nocturnal fish showed a less abrupt response to lights on in the form of decreasing activity over several hours.

Most light-change-active fish (30 of 32) exhibited a pattern very similar to that depicted by the composite plot (Fig. 2c). The least amount of activity occurred during the 6 hr preceding lights off. An abrupt rise at lights off was followed by

an intermediate level of activity during the dark interval. A second burst at lights on was followed by a higher level of activity for the next 6 hr. The other two fish in this group had high levels of activity only at times of light change and were relatively inactive at other times. Two fish showed some evidence of anticipating lights on by an increase in activity during the 2 hr preceding the transition.

The periodogram of light-change fish contained distinct secondary maxima at the period length of 12.0 hr (Fig. 1b). The periodograms of nocturnal or diurnal fish occasionally showed secondary peaks which were less distinct. Some diurnal fish showed recurring bursts of activity shortly after midnight which lasted for one or two hours.

Four LD experiments were carried out during the summer of 1972. Most fish (30 of 42) were diurnal (Table I). Eleven fish were light-change-active and only one was nocturnal. In contrast, Atlantic salmon tested during the previous summer in LD cycles of length approximating the natural cycle were about equally distributed among the three activity patterns (Varanelli and McCleave, 1974). Four additional LD experiments of the present study took place during the winter and spring of 1973. In these experiments 19 of 46 fish were nocturnal, 21 were light-change-active and five were diurnal. The trend was from mostly nocturnal activity in the winter to more light-change activity in the spring.

Constant conditions

Fish subjected to LL or DD were similar in being generally aperiodic (Fig. 1c). Intermediate levels of activity were distributed fairly equally over the day in the form of sporadic bursts. Five fish (of 30) in DD produced periodograms which had peak amplitudes at period values near 24.0 hr (23.5–24.2 hr) (Fig. 1d). In only two of these cases could a pattern be found by visual inspection of the daily plots. These two series of plots suggested recurring peaks near subjective evening. No fish in LL showed any evidence of periodicity.

The mean of the total activity counts was computed for each group of experimental fish. The mean from the LL experiments (36,670) was about twice as large as the mean from DD (17,240). The mean from LL experiments was close to the LD mean (31,100). The highest mean total activity (45,000) came from fish subjected to 1 hr of light (LD 1:22).

Short signal entrainment

Twelve fish were exposed to a 1 hr light stimulus beginning every 23 hr for 10 days in an attempt to initiate a 23 hr rhythmicity of activity. Most fish (11 of 12) were aperiodic. In one case a maximum amplitude occurred at 24.0 hr, but no pattern was obvious from the plotted data.

DISCUSSION

Locomotor activity patterns of juvenile Atlantic salmon were entrained by an artificial light-dark cycle. The fact that about one third of the fish had activity induced at times of light change, while the other two thirds were divided between dark-active and light-active suggests that the transition stimulus rather than the

light intensity may be of primary importance in synchronizing the activity rhythms of these fish.

Fish in this study generally did not show an anticipatory change in activity prior to the onset of light or darkness. Byrne (1968) reported that sockeye salmon showed a "pre-dawn" increase and a "pre-dusk" decrease in activity. Pre-dark increases in activity have been observed in the nocturnal swell shark (Nelson and Johnson, 1970) and in juvenile Atlantic herring (Stickney, 1972).

Atlantic salmon showed a consistently greater response following a light to dark transition than following a dark to light change (Fig. 2). This was true even of light-active fish. Maximum activity in juvenile herring occurs at sunset at a time of critical light intensity (Stickney, 1972). Sunset is the primary synchronizer of activity rhythms of two Arctic sculpins *Cottus gobio* and *Cottus poecilopus* (Andreasson, 1969, 1973). In contrast, maximum bursts of activity occur at sunrise in brown trout, *Salmo trutta*, (Swift, 1964), the blenny, *Coryphoblennius galerita*, (Gibson, 1970) and bluefish (Olla and Studholme, 1972).

The nature of light responses in juvenile Pacific salmon is variable and complex (Hoar, Keenleyside and Goodall, 1957). Although different species exhibited preferences for either bright areas (*Onchorhynchus keta, O. gorbuscha*) or dark areas (*O. kisutch, O. nerka*), all individuals continually ventured into both light and dark regions of the test apparatus.

Juvenile Pacific salmon also change their light responses during development. Byrne (1968) found that sockeye salmon are nocturnal for the first two weeks after emergence and then become diurnal. Pink salmon fry, *O. gorbuscha*, lose their photonegative behavior after two months (Hoar, Keenleyside and Goodall, 1957). Coho salmon fry, *O. kisutch*, are indifferent to light, but the smolts are photonegative. Coho and sockeye salmon smolts are more light sensitive than the fry and seek out deeper darker areas (Hoar, Keenleyside and Goodall, 1957).

Byrne (1968) found that LL facilitated the expression of a free-running rhythm in sockeye salmon whereas DD inhibited it. This is opposite to what Ali (1964) found for Atlantic salmon. Gibson (1971) entrained rhythms in the blenny, *Blennius pholis*, that persisted in DD after several months of exposure to LD. Another blenny, *Coryphoblennius galerita*, exhibited a 12 hr tidal activity rhythm in DD (Gibson, 1970). Nelson and Johnson (1970) found that neither LL nor DD inhibited the free-running rhythm of the swell shark for about 15 days. Several fish having indications of rhythmicity in DD were noted by us and by Varanelli and McCleave (1974).

The majority of fish in some experiments was diurnal while in others it was nocturnal or light-change-active. Also, different individuals tested simultaneously in the same conditions showed different patterns of activity. Varanelli and McCleave (1974) found that slightly more Atlantic salmon tested during the summer of 1971 were nocturnal than were diurnal or light-change-active. In contrast, we found that during the following summer most fish were diurnal. During the winter and spring of 1973 the majority of fish were nocturnal or light-change-active and very few were diurnal (Table I). Because Varanelli and McCleave (1974) used various photoperiods and temperatures to approximate environmental conditions, it is possible that some interaction among photoperiod, light intensity and temperature was responsible for the difference in results between the two summers.

This does not explain why different experiments with the same conditions produced different results or why fish tested at the same time showed different behavior patterns.

Byrne (1968) found that a photoperiod-temperature interaction was responsible for changes in the activity patterns of juvenile sockeye salmon. While testing fish in several photoperiod-temperature combinations, he found that an upper temperature limit existed for each photoperiod. When this temperature was exceeded the fish changed from light-active to dark-active. High temperatures (10° C) coupled with short photoperiods (LD 8:16) caused an increase in nocturnal activity.

Seasonal changes in activity pattern have been found in Arctic populations of *Cottus gobio* and *C. poecilopus* (Andreasson, 1973), brook trout, *Salvelinus fontinalis* (Eriksson, 1972), and burbot, *Lota lota,* (Müller, 1973). The trout is day-active all year except for a desynchronized interval in summer. The sculpins and burbot are day-active in winter and night-active in summer (Andreasson, 1973). During the phase shift *C. poecilopus* passes through an interval when peak activity occurs at sunrise and sunset. Andreasson (1973) suggested that the activity rhythms of these fish are controlled by two separate oscillators, one light-active and one dark-active. A seasonal change in the phase angle between the two oscillators, caused by exogenous factors, is responsible for the change in activity pattern.

Müller (1973) found that the duration of activity in burbot in the Arctic is controlled by day length in winter and by the night length in summer. The activity time cannot exceed 10–11 hr. When the day length or night length exceeds this limit, the fish switches to nocturnal or diurnal activity, respectively.

The phase of a circadian rhythm can sometimes be shifted by a single perturbation in the light, temperature, or other regime. A single chemical stimulation via trout scent initiated a circadian activity rhythm in the adult sea lamprey which persisted in constant dim light until observations were stopped after five days (Kleerekoper, Taylor and Wilton, 1961). Fish in the present study failed to become entrained by an hour long light signal recurring periodically every 23 hr. Neither did they show the abrupt increase in activity following the light to dark transition observed in the LD experiments. Maximum outbursts of activity did not coincide with the hour of light exposure.

The hour of light exposure began in the evening and occurred an hour earlier each day over the course of the experiment (ten days). This signal may have been presented at a point in a rhythm of light sensitivity at which it could not be effective. Bruce (1960) has generalized that a light presented during an animal's subjective day may cause little or no phase shift. Because these fish have shown abrupt responses to LD transitions in other experiments, the synchronizing regime applied in this case was probably inappropriate. More information is needed about the variation in light responses and the limits of entrainability of salmonids to clarify the phase relationships between their activity rhythms and the oscillations of their environment. Indicator processes other than locomotor activity may be better suited for the demonstration of endogenous or non-visual exogenous components of circadian rhythms.

Summary

Atlantic salmon parr exposed to a 12 hr light–12 hr dark cycle (LD 12:12) for ten days were entrained to a 24.0 hr periodicity in locomotor activity. Thirty-five fish were light-active, 20 were dark-active and 32 were active primarily when lights were turned on or off. Fish maintained in constant conditions (75) were generally aperiodic. Five fish (of 30) in constant darkness (DD) showed evidence of 24.0 hr periodicity. Twelve fish exposed to a light signal of 1 hr duration recurring every 23 hr failed to become entrained. Fish in constant light (LL) showed more activity than fish in DD. The results suggest (1) that light-dark transitions are important in synchronizing locomotor activity rhythms and (2) that locomotor activity is not a good indicator of possible circadian oscillations in this species.

Literature Cited

Ali, M. A., 1964. Diurnal rhythms in the rates of oxygen consumption, locomotor and feeding activity of yearling Atlantic salmon (*Salmo salar*) under various light conditions. *Proc. Indian Acad. Sci. Section B*, **60**: 249–263.

Andreasson, S., 1969. Locomotory activity patterns of *Cottus poecilopus* Heckel and *Cottus gobio* L. (Pisces). *Oikos*, **20**: 79–94.

Andreasson, S., 1973. Seasonal changes in diel activity of *Cottus poecilopus* and *Cottus gobio* (Pisces) at the Arctic Circle. *Oikos*, **24**: 16–23.

Aschoff, J., 1960. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harbor Symp. Quant. Biol.*, **25**: 11–28.

Aschoff, J., 1966. Circadian activity with two peaks. *Ecology*, **47**: 657–672.

Bruce, V. G., 1960. Environmental entrainment of circadian rhythms. *Cold Spring Harbor Symp. Quant. Biol.*, **25**: 29–47.

Byrne, J. E., 1968. The effects of photoperiods and temperature on the daily patterns of locomotor activity in juvenile sockeye salmon (*Oncorhynchus nerka*). Ph.D. thesis, University of British Columbia, Vancouver, 125 p.

Enright, J. T., 1965. Accurate geophysical rhythms and frequency analysis. Pages 31–42 in J. Aschoff, Ed., *Circadian Clocks*. North Holland Publishing Co., Amsterdam.

Eriksson, L., 1972. Die Jahresperiodik augen-und pinealorganloser Bachsaiblinge *Salvelinus fontinalis* Mitchell. *Aquilo Ser. Zool.*, **13**: 8–12.

Gibson, R. N., 1970. The tidal rhythm of activity of *Coryphoblennius galerita* L. (Teleostei, Blennidae). *Anim. Behav.*, **18**: 539–543.

Gibson, R. N., 1971. Factors affecting the rhythmic activity of *Blennius pholis* L. (Teleostei). *Anim. Behav.*, **19**: 336–343.

Hoar, W. S., M. H. A. Keenleyside and R. G. Goodall, 1957. Reactions of juvenile Pacific salmon to light. *J. Fish. Res. Board Can.*, **14**: 815–830

Kleerekoper, H., G. Taylor and R. Wilton, 1961. Diurnal periodicity in the activity of *Petromyzon marinus* and the effects of chemical stimulation. *Trans. Amer. Fish. Soc.*, **90**: 73–78.

Lissmann, H. W., and H. O. Schwassmann, 1965. Activity rhythm of an electric fish, *Gymnorhamphichthys hypostomous* Ellis. *Z. Vergl. Physiol.*, **51**: 153–171.

Müller, K., 1973. Seasonal phase shift and the duration of activity time in the burbot *Lota lota*. *J. Comp. Physiol.*, **84**: 357–359.

NELSON, D. R., AND R. H. JOHNSON, 1970. Diel activity rhythms in the nocturnal bottom-dwelling sharks *Heterodontus francisci* and *Cephaloscyllium ventriosum.* *Copeia,* **1970**: 732–739.

OLLA, B. L., AND A. L. STUDHOLME, 1972. Daily and seasonal rhythms of activity in the bluefish (*Pomatomus saltatrix*). Pages 303–325 in H. E. Winn and B. L. Olla, Eds., *Behavior of Marine Animals, Vol. 2, Vertebrates.* Plenum Press, New York.

SCHWASSMANN, H. O., 1971. Biological rhythms. Pages 371–416 in W. S. Hoar and D. J. Randall, Eds., *Fish Physiology,* Vol. 6. Academic Press, New York.

SEIGMUND, R., AND D. L. WOLFF, 1973. Circadian-Rhythmik und Gruppenverhalten bei *Leucaspius delineatus. Experientia,* **29**: 54–58.

STICKNEY, A. P., 1972. The locomotor activity of juvenile herring (*Clupea harengus*) in response to changes in illumination. *Ecology,* **53**: 438–445.

SWIFT, D. R., 1964. Activity cycles in the brown trout (*Salmo trutta*) 2. Fish artificially fed. *J. Fish. Res. Board Can.,* **21**: 133–138.

VARANELLI, C. C., AND J. D. McCLEAVE, 1974. Locomotor activity of Atlantic salmon parr (*Salmo salar*) in various light conditions and in weak magnetic fields. *Anim. Behav.,* **22**: 178–186.